

CLAIMS

1. A modulator of the expression, proteolytic processing, bioactivity or intracellular signaling of the 26 kDa cell-surface-bound form of TNF (26 kDa TNF), said modulator being capable of interacting with the intracellular domain of said 26 kDa TNF or with one or more other intracellular effector proteins which interact with said intracellular domain of the 26 kDa TNF.
2. A modulator according to claim 1, wherein said modulator is selected from the group comprising : (i) naturally-derived proteins, peptides, analogs and derivatives thereof capable of interacting with said intracellular domain of 26 kDa TNF or with said other intracellular effect proteins; (ii) synthetically produced complementary peptides synthesized by using as substrate the intracellular domain or portions thereof of the 26 kDa TNF, said complementary peptides being capable of interacting with said intracellular domain of the 26 kDa TNF or with said other intracellular effector proteins; (iii) antibodies or active fragments thereof capable of interacting with said intracellular domain of the 26 kDa TNF or with said other intracellular effector proteins; and (iv) organic compounds capable of interacting with said intracellular domain of the 26 kDa TNF or with said other intracellular effector proteins, said organic compounds being derived from known compounds and selected using said intracellular domain or portions thereof of 26 kDa TNF as a substrate in a binding assay, or being synthesized using said intracellular domain or portions thereof of 26 kDa TNF as a substrate for designing and synthesizing said organic compounds.
- A 3. A modulator according to claim 1 ~~or claim 2~~, wherein said modulator is capable of interacting with one or more serine residues in the intracellular domain of said 26 kDa TNF which are substrates of phosphorylation, or with one or more phosphorylated serine residues in the intracellular domain of said 26 kDa TNF, or with one or more kinase enzymes which are involved in the phosphorylation of said one or more serine residues, or with one or more other intracellular effector proteins which interact with said serine or phosphorylated serine residues.
4. A DNA sequence encoding a modulator being a protein, peptide or an analog thereof, according to ^{claim 1} ~~any one of claims 1-3~~.

5. A DNA sequence according to claim 4 encoding a naturally-derived protein or peptide selected from the group consisting of :
 - (a) a cDNA sequence derived from the coding region of a native 26 kDa TNF intracellular domain-binding protein or peptide;
 - 5 (b) DNA sequences capable of hybridization to a sequence of (a) under moderately stringent conditions and which encode a biologically active 26 kDa TNF intracellular domain-binding protein or peptide; and
 - (c) DNA sequences which are degenerate as a result of the genetic code to the DNA sequenced defined in (a) and (b) and which encode a biologically active 26 kDa TNF intracellular domain-binding protein.
- 10 6. A protein, peptide or analogs thereof encoded by a DNA sequence according to claim 4 or claim 5, said protein, peptide and analogs being capable of binding to or interacting with the intracellular domain of the 26 kDa TNF.
7. A vector comprising a DNA sequence according to claim 4 or claim 5.
- 15 8. A vector according to claim 7 which is capable of being expressed in a eukaryotic or prokaryotic host cell.
9. Transformed eukaryotic or prokaryotic host cells containing a vector according to claim 8.
- 20 10. A method for producing the protein, peptide or analogs according to claim 6 comprising growing the transformed host cells according to claim 9 under conditions suitable for the expression of said protein, peptide or analogs, effecting post-translational modifications of said protein, peptide or analogs as necessary for the obtention thereof and extracting said expressed protein, peptide or analogs from the culture medium of said transformed cells or from cell extracts of said transformed cells.
- 25 11. Antibodies or active fragments or derivatives thereof specific for the protein, peptide or analogs according to claim 6.
12. A method for the modulation of the expression, proteolytic processing, bioactivity or intracellular signaling of the 26 kDa TNF comprising treating cells with a modulator according to any one of claims 1-3, or with a protein, peptide or analogs according to claim 6, or with antibodies, active fragments or derivatives according to claim 11;
 - 30 B wherein said treating of cells comprises introducing into the cells said naturally derived modulator is

- ~~B proteins, peptides, analogs and derivatives thereof, said complementary peptides, said~~
~~B antibodies, or said organic compounds~~ in a form suitable for intracellular introduction
~~B thereof, or when said modulator is a protein, peptide or analog thereof, said treatment~~
 of cells also comprises introducing into said cells a DNA sequence encoding said protein,
 5 ~~B peptide or analog thereof~~ in the form of a suitable vector carrying said sequence, said vector
 being capable of effecting the insertion of said sequence into said cells in a way that said
 sequence is expressed in the cells.
- ~~B 13. A method according to claim 12, wherein said treating of cells is by administration of~~
~~B said protein, peptide or analogs, and said administration is by transfection of said cells~~
 10 with a recombinant animal virus vector comprising the steps of :
 (a) constructing a recombinant animal virus vector carrying a sequence encoding a viral
 surface protein (ligand) that is capable of binding to a specific cell surface receptor of the
~~B surface of said cell to be treated, and a second sequence encoding a protein, peptide or~~
~~B analogs thereof according to any one of claims 2, 3 and 6, said protein, peptide or~~
 15 ~~B analogs~~ when expressed in said cells, being capable of modulating the expression,
 proteolytic processing, bioactivity or intracellular signaling of the 26 kDa TNF by
 interacting with the intracellular domain of said 26 kDa TNF or by interacting with
 another intracellular effector protein which interacts with said 26 kDa TNF intracellular
 domain; and
 20 (b) infecting said cells with said vector of (a).
- ~~B 14. A method according to claim 12, wherein said treating of cells is by administration of~~
~~B said antibodies, active fragments or derivatives thereof according to any one of claims 2,~~
~~B 3 and 11, said treating being by application of a suitable composition containing said~~
 25 antibodies, active fragments or derivatives thereof to said cells, said composition being
 formulated for intracellular application.
15. A method for the modulation of the expression, proteolytic processing, bioactivity or
 intracellular signaling of the 26 kDa TNF in 26 kDa TNF-producing cells, comprising
 treating said cells with an oligonucleotide sequence selected from a sequence encoding
~~A an antisense sequence of at least part of the sequence according to claim 4 or claim 5,~~
 30 said oligonucleotide sequence being capable of blocking the expression of at least one
 protein or peptide which interacts with the intracellular domain of the 26 kDa TNF.

but, 62
16. A method according to claim 15, wherein said oligonucleotide sequence is introduced into said cells via a recombinant virus vector of claim 13, wherein said second sequence of the virus encodes said oligonucleotide sequence.

5 17. A method for modulation of the expression, proteolytic processing, bioactivity or intracellular signaling of the 26 kDa TNF in 26 kDa TNF-producing cells, comprising applying the ribozyme procedure in which a vector encoding a ribozyme sequence capable of interacting with a cellular mRNA sequence encoding a protein or peptide according to claim 6, is introduced into said cells in a form that permits expression of
10 said ribozyme sequence in said cells and wherein, when said ribozyme sequence is expressed in said cells it interacts with said cellular mRNA sequence and cleaves said mRNA sequence resulting in the inhibition of expression of said protein or peptide in said cells.

15 18. A method for isolating and identifying proteins, peptides, factors or receptors capable of interacting with or binding to the intracellular domain of the 26 kDa TNF comprising applying the procedure of affinity chromatography in which the intracellular domain or portions thereof of the 26 kDa TNF is attached to the affinity chromatography matrix and is brought into contact with a cell extract, and proteins, peptides, factors or receptors from the cell extract which bound to said attached 26 kDa TNF intracellular domain or portions thereof, are then eluted, isolated and analyzed.

20 19. A method for isolating and identifying proteins and peptides capable of binding to the intracellular domain of the 26 kDa TNF comprising applying the yeast two-hybrid procedure in which a sequence encoding said 26 kDa TNF intracellular domain or portions thereof is carried by one hybrid vector and a sequence from a cDNA or genomic DNA library are carried by the second hybrid vector, the vectors then being
25 used to transform yeast host cells and the positive transformed cells being isolated, followed by extraction of said second hybrid vector to obtain a sequence encoding a protein or peptide which binds to said 26 kDa TNF intracellular domain or portions thereof.

30 20. A method for isolating and identifying a protein or peptide capable of binding to the intracellular domain of the 26 kDa TNF comprising applying the procedure of non-stringent Southern hybridization followed by PCR cloning in which a sequence or parts

A thereof according to claim 4 ~~or claim 5~~ is used as a probe to bind sequences from a cDNA or genomic DNA library having at least partial homology thereto, said bound sequences then being amplified and cloned by the PCR procedure to yield clones encoding proteins or peptides having at least partial homology to said sequences of claim 4 ~~or claim 5~~.

5 A 21. A pharmaceutical composition for the modulation of the expression, proteolytic processing, bioactivity or intracellular signaling of the 26 kDa TNF comprising, as active ingredient, a modulator according to any one of claims 1-3, or a protein, peptide or analogs thereof according to claim 6, or antibodies, active fragments or derivatives thereof according to claim 11, and a pharmaceutically acceptable excipient, carrier or diluent.

10 22. A pharmaceutical composition for the modulation of the expression, proteolytic processing, bioactivity or intracellular signaling of the 26 kDa TNF comprising, as active ingredient, a recombinant animal virus vector encoding a protein capable of binding a cell surface receptor and encoding a protein or peptide or ^{analog} ~~analog~~ thereof according to claim 6.

15 23. A pharmaceutical composition for the modulation of the expression, proteolytic processing, bioactivity or intracellular signaling of the 26 kDa TNF comprising, as active ingredient, an oligonucleotide sequence encoding an anti-sense sequence of the sequence according to claim 4 ~~or claim 5~~.

20 24. ~~A method for designing drugs that are capable of modulating the expression, proteolytic processing, bioactivity or intracellular signaling of the 26 kDa TNF comprising the procedures described herein in Examples 6 and 7.~~